Cultivation and Growth of Microorganisms

Overview

- Much of what has been learned in microbiology has come from the cultivation of microorganisms in the laboratory.
- As we have already learned, microorganisms are cultivated on media, which provide nutrients.
- In addition, the proper physical environment must be provided for optimal growth.
- Some of the species grow at temperature near the freezing point of water; others grow at temperature as high as 55°C.
- Oxygen is essential to some, poisonous to others.
- Most bacteria grow best at or near neutral pH, but the tolerable range of pH for growth among microbes varies from alkaline to acidic.
- Thus physical conditions must be adjusted in the laboratory to meet the special growth needs of specific species.

Overview

- Once the chemical and physical requirements are satisfied, it is possible to study the mode of reproduction and growth of a species of microorganism.
- Eucaryotes and procaryotes differ in their methods of reproduction. For example, eucaryotes have developed elaborate processes to ensure that each daughter cell receives the correct number of chromosomes following sexual reproduction.
- However, it is important to remember that the behavior of a species in pure culture in the laboratory may not be same as its growth characteristics in nature.
- Pure culture in the laboratory are pampered because they usually have an overabundance of nutrients and don't have to compete with other microbes for available food.

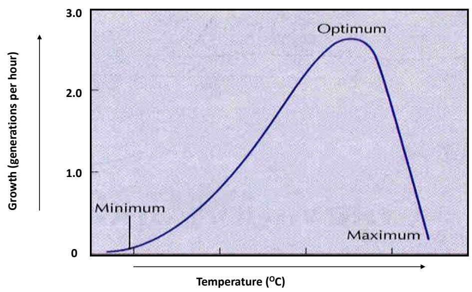
Physical conditions for Cultivation of Microorganisms

- Four main conditions influence the physical environment of a microbe: temperature, pH, gaseous atmosphere, and osmotic pressure.
- The successful cultivation of the various types of microorganisms requires a combination of the proper nutrients and the proper physical environment.
- Microbiologists must know what a specific microbe requires for growth, satisfy those needs, and check the cultures to make certain that the organisms are thriving.

Physical conditions for Cultivation of Microorganisms

- Temperature:
  - Temperature has a great influence on the growth of microorganisms. This is not surprising, since all the processes of growth are dependent on the chemical reactions that are affected by temperature.
  - For any particular microbe, the three important temperatures are the minimum, optimum, and maximum growth temperatures.
  - These are known as the cardinal temperatures of a species of microorganism.

Typical growth response of a microorganism to incubation temperatures, showing minimum, optimum, and maximum temperatures

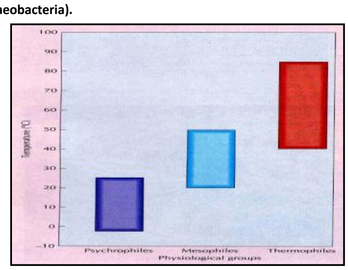


Physical conditions for Cultivation of Microorganisms

- Temperature:
  - In addition to affecting the growth rate, temperature may also affect the type of reproduction, morphology, metabolic process, and nutritional requirements.
  - Therefore, the optimum temperature for the growth may not necessarily be the optimum temperature for every cellular activity.
  - Microorganisms may be divided into three groups on the basis of the temperature range in which they grow best.
    1. Psychlorphiles, or cold-loving microbes
    2. Mesophiles, or moderate-temperature – loving microbes
    3. Thermophiles, or heat-loving microbes.

Physical conditions for Cultivation of Microorganisms

Approximate temperature ranges for growth of various physiological groups of microorganisms (excluding the extreme thermophilic archaeobacteria).



Physical Conditions for Cultivation of Microorganisms

- Temperature:
  1. Psychrophiles:

     Psychrophiles grow best at temperatures from 15 to 20 °C, although they can grow at lower temperatures. Some of these microbes die if they are exposed to room temperatures (about 25 °C) for a short time.
  2. Mesophiles:

     Most microorganisms are mesophiles, growing best within temperature range of 25 to 40 °C. Saprophytic bacteria, fungi, algae, and protozoa grow in the lower part of the mesophilic temperature range.

     Parasitic microorganisms of human and animals grow in the upper part of this range. Those that are pathogenic for the human grow best at about body temperature, which is 37 °C, the elevated temperature of a fever may inhibit the growth of some pathogens.
  3. Thermophiles:

     Most thermophiles grow at the temperatures from about 40 to 85 °C, but they grow best between 50 and 60 °C. These hardy microbes may be found in volcanic areas, compost heaps, and hot springs.

     Most thermophilic microorganisms are procaryotes; no eurocarotic cells are known to grow at temperature greater than 60 °C.

Physical Conditions for Cultivation of Microorganisms

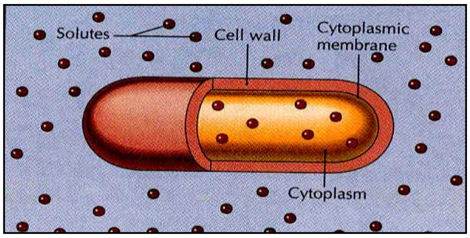
- Gaseous Atmosphere:
  - Microorganisms in their natural habitats require varying amounts of gases such as oxygen, carbon dioxide, nitrogen, and methane.
  - Some gases are used in cellular metabolism; others may have to be excluded from a culture because they are toxic to the cells.
  - On the basis of their response to the gaseous oxygen, microorganisms are divided into four physiological groups:
    1. Aerobes
    2. Facultative microorganisms,
    3. Anaerobes and
    4. Microaerophiles

Physical Conditions for Cultivation of Microorganisms

- Other Conditions:
  - Osmotic pressure is the force with which water moves through the cytoplasmic membrane from a solution containing a low concentration of dissolved substances (solutes) to one containing a high solute concentration.
  - When microbial cells are in aqueous medium, there should not be large differences between solute concentrations inside and outside the cells, or the cells could either dehydrate or lyse.
  - Hydrostatic pressure may also influence microbial growth. This is the pressure exerted on the cells by the weight if the water resting on the top of them.
  - Microorganisms have been isolated from ocean floors that are over 2500 m below sea level, where the pressure is more than 250 bars (250 times atmospheric pressure)
  - These organisms will not grow in the laboratory unless the medium is under similar pressure. Pressure dependent microbes are called barophiles.

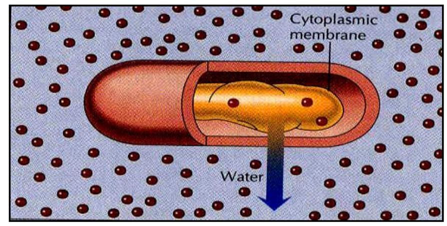
Effect of osmotic pressure on a microbial cell.

Cell in isotonic medium. Concentration of solutes in environment is equal to that within the cell. There is no net movement of water into or out of the cell.



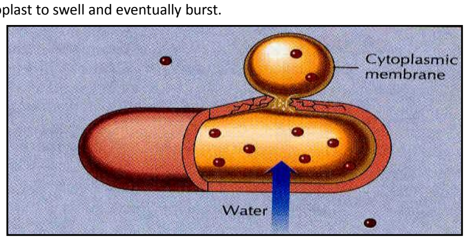
Effect of osmotic pressure on a microbial cell

Cell in hypertonic medium. Concentration of solutes in environment is greater than that within the cell. Water flows out of the cell, resulting in dehydration and shrinking of the protoplast. Cell growth is inhibited; cell may die.



Effect of osmotic pressure on a microbial cell

Cell in hypotonic medium. Concentration of solutes in environment is lower than that within the cell. Water flows into the cell. Net influx of water forces the protoplast against the cell wall. If the wall is weak, it may break, allowing the protoplast to swell and eventually burst.



Reproduction and Growth of Microorganisms

- Growth in a microbial culture usually means an increase in the total number of cells due to reproduction of individual organisms in the culture.
- Therefore there are two phenomena at work:
  - The growth, or reproduction of individual cells ; and
  - The growth, or increase in population of a microbial culture.

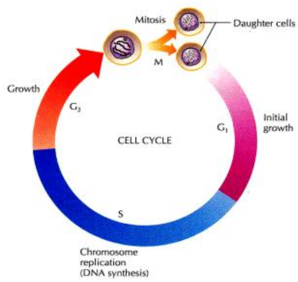
Reproduction and Growth of Microorganisms

- Reproduction in Eucaryotic Microorganisms
  - One of the criteria that define life is that an organism has the capacity to produce others of its kind.
  - In nature, reproduction occurs in two forms:
    - Asexual reproduction and
    - Sexual reproduction
  - Asexual reproduction basically results in new cells identical to the original, while sexual reproduction allows for exchange of genetic material and thus unique offspring.
  - Among eucaryotic microorganisms, both types of reproduction occur, but both are preceded by processes that determine the number of chromosomes involved.

Reproduction and Growth of Microorganisms

- Reproduction in Eucaryotic Microorganisms
  - Asexual Reproduction
    - Asexual reproduction does not involve the union of nuclei, sex cells, or sex organs.
    - It does not provide the opportunity for genetic variation, but it is more efficient than sexual reproduction in propagating a species.
    - In asexual reproduction, new individuals are produced by one parent organism, or, in the case of unicellular organisms, by one cell.
    - Bacteria reproduce asexually by binary fission, in which a parent cell simply splits into two identical daughter cells.
    - Asexual reproduction of eucaryotic microorganisms is more complicated, because it must be preceded by mitosis.
    - Mitosis is a form of nuclear division in which the cell's entire set of chromosomes is duplicated and the two new sets separate to form two identical daughter nuclei.
    - The cell then divides into two daughter cells, each receiving one of the nuclei.
    - Between mitoses, cell are said to be in the "resting" stage with respect to nuclear division; this is called the interphase.

The eucaryotic cell cycle. The S phase is started by the initiation of DNA replication; the initiation of mitosis starts in the M phase. Phase G_1 follows mitosis, and phase G_2 follows DNA synthesis.



Cell Life Cycle:

Interphase – Growth G1; DNA Synthesis S; Growth G2

Mitosis – Prophase (Early, Mid and Late); Metaphase; Anaphase; Telophase

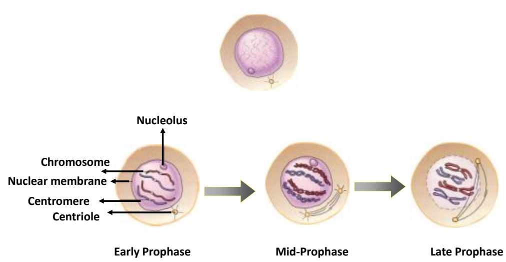
Karyokinesis

Cytokinesis

Reproduction and Growth of Microorganisms

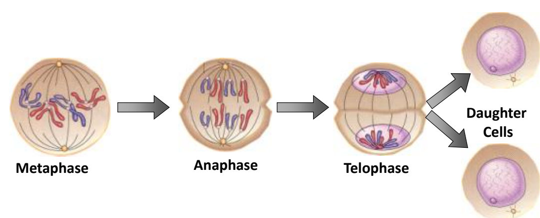
- Reproduction in Eucaryotic Microorganisms
  - Asexual Reproduction
    - It is during a particular period in the interphase that the chromosomes are duplicated.
    - However, the two duplicates do not separate until later, after mitosis begins.
    - For descriptive purposes, the processes of mitosis may be divided into four phases:
      1. Prophase
      2. Metaphase
      3. Anaphase and,
      4. Telophase

The four stages of mitosis: prophase, metaphase, anaphase, and telophase. The DNA is duplicated before mitosis begins, but in early prophase the chromosomes do not look doubled. By mid-prophase, however, the chromosomes do appear doubled.



Reproduction and Growth of Microorganisms

At metaphase the chromosomes align themselves in a plane and attach to mitotic spindle fibers. During anaphase the chromosomes separate and move to opposite poles of the cell. By the end of telophase there are two daughter cells, each of which contains a copy of the genetic material of the parent cell.



Reproduction and Growth of Microorganisms

- Reproduction in Eucaryotic Microorganisms
  - Sexual Reproduction

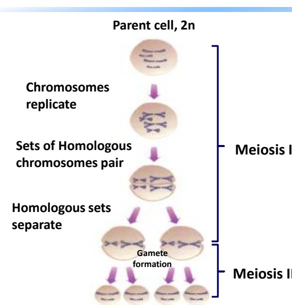
    → Fusion of two different sex cells known as gametes, which are usually from two parents of different sex are mating type.

    → The fusion of gametes is termed fertilization, and the resulting cell is called the zygote.

    → An adult human has approximately 60 trillion ($6x10^3$) cells – all of them derived from asexual reproduction, or mitotic divisions, of the single-celled zygote formed when a sperm and an egg fuse.

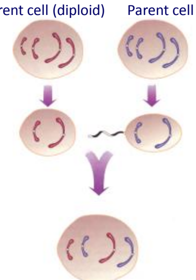
    → Somatic cells – diploid (homologous chromosomes)

Reproduction and Growth of Microorganisms



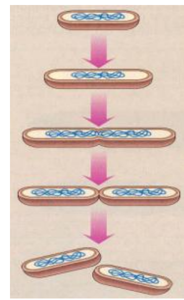
Summary of the main events occurring in meiosis. For simplicity, the parent cell is shown as having only two pairs of chromosomes, one type being shorter than the other. Meiosis may be viewed to occur in two phases, I and II. In meiosis I, the chromosomes of the diploid cell replicate, sets of homologous chromosomes pair, and then the pairs separate. In meiosis II, gametes are formed, each having a haploid set of chromosomes.

Reproduction and Growth of Microorganisms



Overall scheme showing the formation of haploid gametes from diploid parent cells, and the formation of a diploid zygote by the fusion of haploid gametes.

Bacterial multiplication by transverse binary fission



Reproduction and Growth of Microorganisms

- Growth of a Bacterial Culture

  $$1 \rightarrow 2 \rightarrow 4 \rightarrow 8 \rightarrow 16 \rightarrow 32 \rightarrow \ldots\ldots\ldots\ldots$$

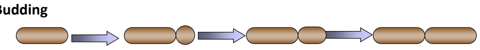
  - This increase may be expressed as a geometric progression in the following manner:

    $$1 \rightarrow 2^2 \rightarrow 2^3 \rightarrow 2^4 \rightarrow 2^5 \rightarrow \ldots\ldots\ldots 2^n$$

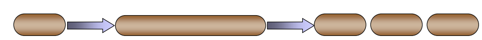
  - Where the exponent "*n*" refers to the number of generations.
  - Generation Time:
    - The time interval required for each microbe to divide, or for the population in a culture to double, is known as generation time.
    - Not all species of microorganisms have the same generation time. For *Escherichia Coli*, the generation time in a rich medium may be as short as 12.5 min.
    - For *Mycobacterium tuberculosis*, it is 13 to 15 h.
    - Nor is the generation time the same for a particular species of microorganism under all conditions.
    - *Escherichia Coli*, for example, will take much longer to divide in a nutritionally poor medium.
    - Generation times are strongly influenced not only by the nutritional composition of the medium, but also by the physical conditions of incubation.

Cell reproduction by unicellular procaryotes other than by transverse binary fission:

Budding

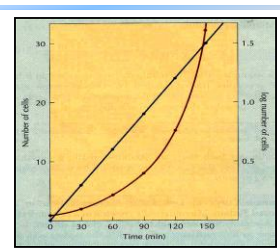


Fragmentation



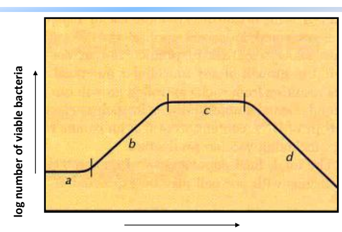
Exospore Formation

Hypothetical bacterial growth curve, assuming that one bacterial cell is inoculated into a medium and divisions occur regularly at 30-min intervals (generation time).



Logarithm of number of bacteria versus time;
Arithmetic number of bacteria versus time.

Microbial Growth in Batch System



Typical bacterial growth curve;
a: lag phase;
b: log (logarithmic), or exponential phase;
c: stationary phase;
d: death or decline phase.

Microbial Growth – Logistic Model:

Let
- $x(t) \rightarrow$ Number of Organisms at time 't'
- $B(x,t) \rightarrow$ Birth (Growth) rate $\rightarrow$ more precisely specific birth (growth) rate
- $D(x,t) \rightarrow$ Death rate $\rightarrow$ more precisely specific death rate

Number of births in time $\Delta t = B(x,t)\, x(t)\, \Delta t$
Number of deaths in time $\Delta t = D(x,t)\, x(t)\, \Delta t$
Any other reason for change in number (population), say

$$\left.\begin{array}{l}\textbf{Immigration} = I(x,t)\, x(t)\, \Delta t \\ \textbf{Emigration} = E(x,t)\, x(t)\, \Delta t\end{array}\right\}$$

Prediction is very complex in nature, several factors influence this.

Microbial Growth – Logistic Model:

Population growth in time Δt

$$= x(t+\Delta t) - x(t) = B(x,t)\, x(t)\, \Delta t + I(x,t)\, x(t)\, \Delta t - D(x,t)\, x(t)\, \Delta t - E(x,t)\, x(t)\, \Delta t$$

or

$$\frac{x(t+\Delta t) - x(t)}{\Delta t} = [B(x,t) + I(x,t)]x(t) - [D(x,t) + E(x,t)]x(t)$$

$\cong$ **B.x – D.x ;** ***neglecting I & E***

Microbial Growth – Logistic Model:

Or as $\Delta t \rightarrow 0$

$$\frac{dx}{dt} = Bx - Dx\ ;$$
$$B = \lambda_1 - \lambda_2 x\ ,$$
$$D = \mu_1 - \mu_2 x$$

B→ Decreasing rate of increase in population
D→ Increasing rate of decrease in population

$$\frac{dx}{dt} = (\lambda_1 - \lambda_2 x)x - (\mu_1 + \mu_2 x)x = (\underbrace{\lambda_1 - \mu_1}_{a})x - (\underbrace{\lambda_2 - \mu_2}_{b})x^2$$

$$= ax - bx^2$$

$$or \quad \frac{dx}{ax - bx^2} = dt \qquad\qquad or \quad \frac{dx}{ax} + \frac{b}{a}\frac{dx}{(a-bx)} = dt$$

Microbial Growth – Logistic Model:

$$or \quad \frac{1}{a}\int\frac{dx}{x} + \frac{b}{a}\int\frac{dx}{a-bx} = \int dt$$

$$or \quad \ln x - \ln(a - bx) = at + const$$

$$at \quad t = 0, x = x_o \Rightarrow const = \ln\frac{x_o}{a - bx_o}$$

$$\therefore \quad \ln\left[\frac{x}{a-bx}\right] = at + \ln\left[\frac{x_o}{a-bx_o}\right]$$

Microbial Growth – Logistic Model:

$$x = \frac{\left(a/b\right)x_o}{x_o + \left(a/b - x_o\right)e^{-at}} \qquad a/b = \omega$$

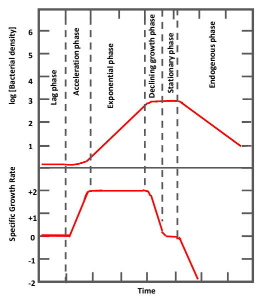
$$x = \frac{\omega\, x_o}{x_o + (\omega - x_o)\, e^{-at}}\ ; \quad x_{max} = \omega \quad i.e.\ at\ \ t = \infty$$

Point of inflection

$$\frac{d}{dt}\left(\frac{dx}{dt}\right) = 0 \Rightarrow \left\{\frac{1}{a}\ln\left[\frac{\omega - x_o}{x_o}\right], \frac{\omega}{2}\right\}$$

Microbial Growth and Substrate Utilization

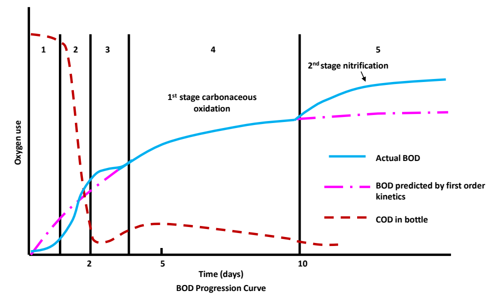
Microbial Growth Curve



Characteristics Growth Curve of Cultures of Microorganisms

- Microbial Number α Microbial Mass, x
- Food or Substrate or Limiting Growth Element, s
- Specific growth rate, dx/dt/x, μ
- Specific substrate utilization rate, q

Microbial Growth



Substrate Utilization and Growth Relationship

Specific Substrate Utilization Rate (q);
Specific Growth Rate (μ) and Yield Coefficient (y)

$$\mu = \frac{\left(dx/dt\right)_g}{x};$$

$$y = \frac{dx}{ds};$$

$$q = \frac{ds/dt}{x} = \frac{\frac{ds}{dt}\cdot\frac{dx}{ds}}{\frac{dx}{ds}x} = \frac{\frac{dx/dt}{x}}{dx/ds} = \frac{\mu}{y}$$

Substrate Utilization and Growth Relationship

Total Substrate Utilized	= Substrate Utilized for Synthesis	+ Substrate Utilized (Oxidized) for Energy

$$(\Delta s)_T = (\Delta s)_S + (\Delta s)_E \quad or \quad \left(\frac{\Delta s}{\Delta x}\right)_T = \left(\frac{\Delta s}{\Delta x}\right)_S + \left(\frac{\Delta s}{\Delta x}\right)_E \quad or \quad \frac{1}{y} = \frac{1}{y_s} + \frac{1}{y_E}$$

y_E is not a real value, it indicates that fraction of 's' removed per unit of 'x' which is channeled into energy metabolism.

$$\left(\frac{\Delta s}{\Delta x}\right)_S = 1 \quad or \quad \frac{1}{y_S} = 1$$

$$(\Delta s)_E = (\Delta s)_{Growth\ Energy} + (\Delta s)_{Maintenance\ Energy} = (\Delta s)_{GE} + (\Delta s)_{ME}$$

$$\left(\frac{\Delta s}{\Delta x}\right)_E = \frac{(\Delta s)_{GE} + (\Delta s)_{ME}}{\Delta x} = \frac{1}{y_E}$$

Substrate Utilization and Growth Relationship

When -

$$(\Delta s)_{ME} = 0, \quad \left(\frac{\Delta s}{\Delta x}\right)_E = \left(\frac{\Delta s}{\Delta x}\right)_{GE} = \frac{1}{y_E}$$

This represents maximum yield condition because a portion of the 's' that might have been oxidized to provide for Maintenance Energy will now be assimilated into new biomass. Under this condition '*y*' is maximum and is termed as true or total growth yield coefficient, "y_T"

$(\Delta s)_{ME} \rightarrow$ Substrate Utilization for Maintenance Energy is proportional to x or,

$$\left(\frac{ds}{dt}\right)_{ME} \propto x \quad or \quad \left(\frac{ds}{dt}\right)_{ME} = bx \rightarrow \text{b Maintenance Energy Coefficient}$$

Substrate Utilization and Growth Relationship

Relationship between S-Utilization, True yield Coefficient and Maintenance Energy (ME) Coefficient

(Assumption: ME requirement satisfied from external substrate):

$$(\Delta s)_{U-T} = (\Delta s)_{U-G} + (\Delta s)_{U-E}$$

$$= (\Delta s)_{U-G} + (\Delta s)_{U-GE} + (\Delta s)_{U-ME}$$

$$= (\Delta s)_{U-GF} + (\Delta s)_{U-ME}$$

Substrate Utilization and Growth Relationship

or in terms of rate:

$$\left(\frac{ds}{dt}\right)_{U-T} = \left(\frac{ds}{dt}\right)_{U-GF} + \left(\frac{ds}{dt}\right)_{U-ME} = \left(\frac{ds}{dt}\right)_{U-GF} + bx$$

$$or \quad \frac{\left(\frac{ds}{dt}\right)_{U-T}}{x} = \frac{\left(\frac{ds}{dT}\right)_{U-GF}}{x} + b = \frac{\frac{1}{y_T}\frac{dx}{dt}}{x} + b\ ;$$

$$\frac{dx}{ds} = y \quad or \quad ds = \frac{dx}{y}$$

$$or \quad q = \frac{\mu}{y_T} + b \quad or \quad \mu = y_T(q-b) \rightarrow (1)$$

Relationship between Substrate Utilization, True yield Coefficient and ME Coefficient in Endogenous respiration

Assumption: When the substrate is completely exhausted i.e. stationary phase/declining growth phase, ME requirement is satisfied through endogenous metabolism i.e. the cellular compounds are oxidized to produce the ME for the cell and hence the biomass decreases (auto-oxidation → expensive in terms of energy yield)

To account for decrease in biomass production that is observed when the specific growth rate, μ, decreases, Herbert (1958) suggested that the ME is satisfied through endogenous metabolisms, i.e. cellular components are oxidized.

Substrate Utilization and Growth Relationship

[Net Growth] = [Total Growth] – [Biomass Lost due to Endogenous Respiration for ME]

$$\left(\frac{dx}{dt}\right)_{N-g} = \left(\frac{dx}{dt}\right)_{T-G} - \left(\frac{dx}{dt}\right)_{ME} \ ; \quad \left(\frac{dx}{dt}\right)_{ME} \propto x = k_d x$$

$$= \left(\frac{dx}{dt}\right)_{T-G} - k_d x$$

$$= y_T\left(\frac{dS}{dt}\right)_{U} - k_d x$$

$$or \quad \frac{\left(\frac{dx}{dt}\right)_{N-g}}{x} = \frac{y_T\left(\frac{ds}{dt}\right)_{U}}{x} - k_d$$

Substrate Utilization and Growth Relationship

$$or \quad \mu = y_T q - k_d \quad or \quad q = \frac{\mu}{y_T} + \frac{k_d}{y_T} \rightarrow (2)$$

Where 'k_d' is microbial decay coefficient or ME coefficient during endogenous respiration → similar to 'b' but not same as 'b'.

$$q = \frac{\mu}{y_T} + b \quad or \quad \mu = y_T(q-b) \rightarrow (1)$$

Compare (1) & (2) → they are similar, $b = {}^{k_d}\!/_{y_T}$ **ho wever specific oxygen utilization will be different.**

$$y_{Observed} \quad or \quad y_{real} \quad or \quad y_{Actual}$$

'y' is a variable depending upon the Growth Stage

Substrate Utilization and Growth Relationship

$$y_{Observed} \quad or \quad y_{real} \quad or \quad y_{Actual}$$

'y' is a variable depending upon the Growth Stage

$$y_{Obs} = \frac{\left({}^{dx}\!/_{dt}\right)_g}{\left({}^{ds}\!/_{dt}\right)_U} = \frac{\frac{\left({}^{dx}\!/_{dt}\right)_g}{X}}{\frac{\left({}^{ds}\!/_{dt}\right)_U}{x}} = \frac{\mu}{q} = \frac{\mu}{\frac{\mu}{y_T} + \frac{k_d}{y_T}} = \frac{y_T}{1 + {}^{k_d}\!/_{\mu}}$$

$$or \quad y_{Obs} = \frac{y_T}{1 + {}^{k_d}\!/_{\mu}} = \frac{y_T}{1 + k_d\theta_c} \rightarrow (3)$$

Process Kinetics

- Understanding chemical process reactions require knowledge of:
  1. Relative equilibrium position of reaction → obtained from chemical thermodynamics.
  2. Rate at which reaction equilibrium is approached → obtained from Chemical Kinetics

  Reaction Rates :

$$\frac{-dCr}{dt} \ or \ \frac{dCp}{dt}$$

$NH_4^+ + 1.5\ O_2 \rightarrow NO_3 + 4H^+$

Process Kinetics

- Relative rate of change for each species is defined by the molar coefficient in the balanced chemical equation
  - The specific numerical value for the rate depends on species considered
  - The overall rate is defined as the rate of change in concentration divided by molar coefficient in balanced equation

$$-\frac{dC_{NH_4}/dt}{1} \quad -\frac{dC_{O_2}/dt}{1.5} \quad +\frac{dC_{NO_3}/dt}{1} \quad +\frac{dC_H/dt}{4}$$

- Most of the kinetic data are analyzed on the basis of the rate of change for a particular chemical species and not in terms of the rate for the overall chemical change

Rate and Order

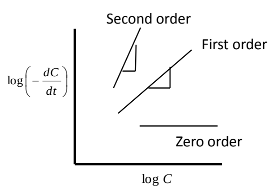
- Chemical reaction may be classified
  - On the basis of stochiometry
  - On a kinetic basis → useful in defining the kinetics
- Classification on the basis of order is generally applicable for
  - Essentially irreversible reactions
  - Initial stages of reversible reactions
  - Reversible reactions, whose position of equilibrium lies far to the right

Reaction Order

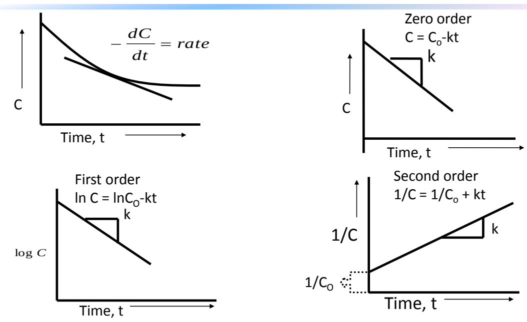
$$-\frac{dC}{dt} = KC^n$$

$$\log\left(-\frac{dC}{dt}\right) = \log K + n \log C$$

- Zero order → $\frac{dC}{dt} = -K$
- First order → $\frac{dC}{dt} = -KC$
- Second order → $\frac{dC}{dt} = -KC^2$
- n^{th} order, etc



Process Kinetics



Reaction Order

- Sum of the exponents on the concentration of the reactants in the differential rate law

$$\frac{dC_A}{dt} = -KC_A(C_B)^2 \rightarrow$$

Third order reaction on the basis of individual components, first order with respect to A and second order with respect to B

Process Kinetics

- Pseudo First Order

$$-\frac{dC_A}{dt} = KC_AC_B \rightarrow very\ high$$

- Rate, Order and Stoichiometry

$$aA + bB \Leftrightarrow cC + dD$$

$$(K_C)_{eq} = \frac{[C]^c[D]^d}{[A]^a[B]^b}$$

Equilibrium Independent of Pathway

- Order of a chemical reaction can not be predicted from stoichiometry
- Exponents in differential rate law not same as stoichiometric coefficients
- n can only be determined experimentally

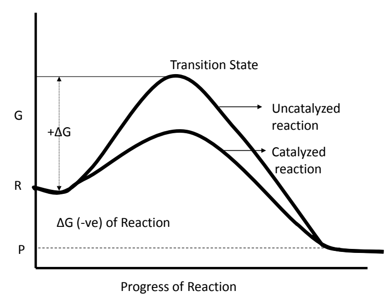
$$A + B \rightarrow P$$

$$-\frac{dC_A}{dt} = kC_A(C_B)^O = -kC_A \qquad -\frac{dC_A}{dt} = kC_AC_B$$

Catalysis

- ΔG indicate spontaneity of the reaction
- Rate of certain spontaneous reaction is very low
  - May take years to detect change in concentration
- Before molecules react, they must pass through a configuration, known as transition state or activated complex
  - Which has energy content greater than the reactions or product
  - Added energy is required → free energy of activation
  - Inversely related to rate of reaction
  - Kinetics depend upon stoichiometry of transition state and not reactant
  - However, in most cases concentration of activated complex is a function of reactant concentration.

Catalyst: Lowers the Activation Energy Does not change



Catalysis

$$A + nH^+ \rightarrow P + nH^+$$

$$-\frac{dC_A}{dt} = K_H[H^+]^n C_A = K_{obs}C_A$$

Catalysis by OH ions

$$A + nOH^- \rightarrow P + n(OH)$$

$$-\frac{dC_A}{dt} = K_{OH}[OH^-]^n C_A = K_{obs}C_A$$

Catalysis

Autocatalysis

$$A + P \rightarrow P + P$$

$$-\frac{dC_A}{dt} = KC_AC_P$$

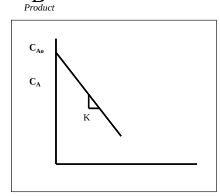
Various Types of Reactions

- **Zero order reaction**
  - **Rate of reaction is independent of the concentration of the reactant**

$$\underset{Reactant}{A} \xrightarrow{k} \underset{Product}{B}$$

$$\frac{d[C_A]}{dt} = -K[C_A] = K$$

$C_A = C_{Ao} - Kt$



Various Types of Reactions

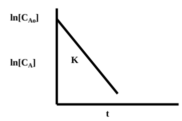
- **<u>First order reaction</u> → rate of reaction is directly proportional to the concentration of the reactant.**

$$\underset{Reactant}{A} \xrightarrow{k} \underset{Product}{B}$$

$$\frac{\partial[CA]}{\partial t} = K[CA]$$

Or

$$\ln[C_A] = \ln[C_{Ao}] - Kt$$



Example:
(1) BOD exertion
(2) Chick's law of disinfection
(Most commonly used /applicable rate of reaction in environmental engineering)

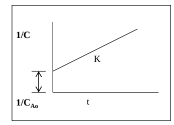
Various Types of Reactions

3. <u>Second order reaction</u> → rate of reaction is proportional to the second power of concentration of reactant.

$$A \xrightarrow{K} B$$

$$\frac{d[C_A]}{dt} = -KC_A^2$$

$$\frac{1}{C_A} - \frac{1}{C_{A_0}} = Kt$$



Microbial growth

$$S + X \rightarrow CO_2 + H_2O + MoreX$$

$$\frac{dS}{dt} = K_1[S][X]$$

$$\frac{dCO_2}{dt} = K_2[S][X]$$

$$\frac{dX}{dt} = K_3[S][X]$$

for CO_2

Various Types of Reactions

4. <u>Consecutive reaction</u> →

$$A \xrightarrow{K_1} B \xrightarrow{K_2} C$$

Decay of Radioactive Elements

$$N_1 \xrightarrow{K_1} N_2 \xrightarrow{K_2} N_3$$

One Radioactive element — Another Radioactive element — Stable product

2. Oxidation of Ammonia to NO_2 to NO_3 by nitrifying organisms

$$NH_3 \xrightarrow[k_1]{O_2} N_2 \xrightarrow[K_2]{O_2} N_3 \ \text{(stable)}$$

Various Types of Reactions

Let us consider,

Also, N_2 is decomposing to N_3 at

$$N_1 \xrightarrow{K_1} N_2 \xrightarrow{K_2} N_3$$

$$-\frac{dN_1}{dt} = k_1 N_1 = rate\ of\ decomposit\ ion$$

$$= \frac{dN_2}{dt} = rate\ of\ fo\ rmation\ of\ N_2$$

$$\frac{dN_2}{dt} = -k_2 N_2$$

$$\frac{dN_1}{dt} = -K_1 N_1$$

$$\Rightarrow N_1 = N_1^0 e^{-K_1 t}$$

$$\frac{dN_2}{dt} = K_1 N_1 - K_2 N_2$$

$$or -\frac{dN_2}{dt} = K_2 N_2 - K_1 N_1^0 e^{-K_1 t} (1)$$

Various Types of Reactions

For this linear equation of first order (first order linear differential equation), assume a solution of the form,

$$N_2 = uv$$

Where, u and v are function of time t.

$$\frac{\partial N_2}{\partial t} = u\frac{\partial v}{\partial t} + v\frac{\partial u}{\partial t}$$

$$u\frac{\partial v}{\partial t} + v\frac{\partial u}{\partial t} + K_2 N_2 - K_1 N_1^0 e^{-K_1 t} = 0$$

$$u\frac{\partial v}{\partial t} + v\frac{\partial u}{\partial t} + K_2 uv - K_1 N_1^0 e^{-K_1 t} = 0$$

$$or,\ u\left(\frac{\partial v}{\partial t} + K_2 v\right) + v\frac{\partial u}{\partial t} - K_1 N_1^0 e^{-K_1 t}$$

$$-\frac{dN_2}{dt} = K_2 N_2 - K_1 N_1^0 e^{-K_1 t} (1)$$

Choose v such that

$$\frac{\partial v}{\partial t} + K_2 v = 0$$

$$\Rightarrow \ell nv = -K_2 t + c$$

$$or\ \ v = e^{-K_2 t + c} = c_1 e^{-K_2 t}$$

Various Types of Reactions

Substituting value of v, we get

$$c_1 e^{-K_2 t}\frac{du}{dt} - K_1 N_1^0 e^{-K_1 t} = 0$$

$$or, \frac{du}{dt} = \frac{K_1 N_1^0}{c_1}\frac{e^{-K_1 t}}{e^{-K_2 t}}$$

$$or, \frac{\partial u}{\partial t} = \frac{K_1 N_1^0}{c_1} e^{(K_2 - K_1)t}$$

$$or, u = \frac{K_1 N_1^0}{c_1(K_2 - K_1)} e^{(K_2 - K_1)t} + \text{constant}$$

$$N_2 = uv = \frac{K_1 N_1^0}{c_1(K_2 - K_1)} e^{(k_2 - k_1)t} c_1 e^{-k_2 t} + \text{constant}\ c_1 e^{-k_2 t};$$

Various Types of Reactions

Boundary condition at t = 0 $N_2 = N_2^0$

$$\Rightarrow c = N_2^0 - \frac{K_1 N_1^0}{K_2 - K_1}$$

$$\therefore N_2 = \underbrace{\frac{K_1}{K_2 - K_1} N_1^0 \left[e^{-K_1 t} - e^{-K_2 t}\right]}_{\text{(Formation of } NO_2 \text{ and conversion of } NO_2 \text{ to } NO_3\text{)}} + \underbrace{N_2^0 e^{-K_2 t}}_{\text{(Conversion of } NO_2 \text{ initially present to } NO_3\text{)}}$$

For concentration of N_3, we can write

$$\frac{\partial N_3}{\partial t} = K_2 N_2 = \frac{K_2 K_1 N_1^0}{K_2 - K_1}\left[e^{-K_1 t} - e^{-K_2 t}\right] + K_2 N_2^0 e^{-K_2 t}$$

$$N_3 = \underbrace{\frac{N_1^0}{K_2 - K_1}\left[K_1 e^{-K_2 t} - K_2 e^{-K_1 t}\right]}_{from\ \mathrm{N}_1^0} + \underbrace{N_2^0\left[1 - e^{-K_2 t}\right]}_{from\ \mathrm{N}_2^0} + \underbrace{N_3^0}_{Initial}$$

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Various Types of Reactions

Similar Differential Equation (Streeter-Phelps Equation) can be derived for DO deficit in streams

$$\frac{\partial D}{\partial t} = K_1 L - K_2 D \Rightarrow \frac{K_1 L_u}{K_2 - K_1}\left[e^{-K_1 t} - e^{-K_2 t}\right] + \underbrace{D_o e^{-K_2 t}}_{\text{(initial DO deficit)}}$$

Reversible Reactions

$$A \underset{k_2}{\overset{k_1}{\leftrightarrow}} B$$

$$\frac{\partial C_A}{\partial t} = -K_1[C_A] + K_2[C_B]$$

First order reversible reaction used in adsorption/ion exchange, etc.
(Basis for Langmuir Adsorption Isotherm equation)

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Various Types of Reactions

Complex Reactions

$$A \underset{}{\overset{K_1}{\longleftrightarrow}} I \overset{K_2}{\longrightarrow} B$$

The net rate of change of I

$$\frac{\partial C_I}{\partial t} = K_1[C_A] - \{K_{-1}(C_I) + K_2(C_I)\}$$

for maximum production of B, there should be no accumulation of I

$$i.e. \frac{\partial C_I}{\partial t} = 0 \Rightarrow K_1[C_A] = (K_{-1} + K_2)C_I$$

A typical example of this type of reaction is <u>Enzyme Substrate Complex Reaction</u>

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Various Types of Reactions

Complex Reaction: Enzyme-Substrate Reaction

$$\underbrace{E + S \underset{k_2}{\overset{k_1}{\Longleftrightarrow}} ES}_{\text{Reversible combination of E \& S to form ES Complex}} \underbrace{\overset{k_3}{\Longrightarrow} E + P}_{\text{Irreversible decomposition of ES to E \& P}}$$

When the concentration of ES complex appears constant a dynamic equilibrium (steady state) condition prevails , where

Rate of complex formation = rate of complex decomposition

$$k_1[E][S] = k_2[ES] + k_3[ES]$$

$$or\ \frac{[E][S]}{[ES]} = \frac{k_2 + k_3}{k_1} = k_m \rightarrow \text{Michaelis constant}$$

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Various Types of Reactions

The maximum reaction rate for formation of product will occur when all E is associated with ES i.e. $R_{max} = K_3[E_{TOTAL}]$

At any other stage , $R = K_3[ES]$

Also,

$$[E_{total}] = [E] + [ES]$$

$$or, [E] = \frac{R_{max}}{k_3} - \frac{R}{k_3}$$

Substituting for "E" from (1), we get

$$\frac{k_m[ES]}{[S]} = \frac{R_{max}}{k_3} - \frac{R}{k_3}$$

$$or, [S]\,[R_{max} - R] = k_m k_3 [ES] = k_m R$$

$$\therefore R = \frac{R_{max}[S]}{k_m + [S]} \quad \text{Michaelis - Menten Equation}$$

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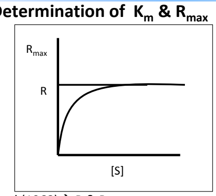
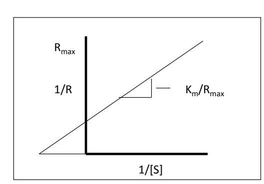
Various Types of Reactions

Determination of K_m & R_{max}





Segel (1968)→ B & R
Zero order S ≥ 100 K_m
First order S ≤ 0.01 K_m
For all practical purposes S ≤ K_m first order may be assumed
Goldman *et al* for all practical purposes S ≤ k_m

$$\frac{1}{R} = \left[\frac{k_m}{R_{max}}\right]\frac{1}{S} + \frac{1}{R_{max}}$$

Line Weaver - Brake Plot

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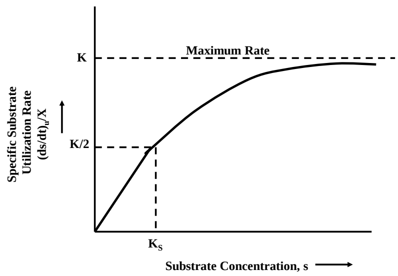
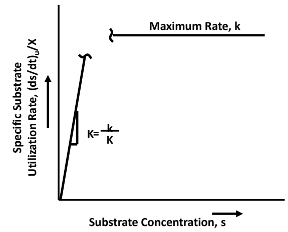
Various Types of Reactions



Various Types of Reactions



Relationship Between Substrate Utilization Rate and Substrate Concentration

Various Types of Reactions

Kinetics: Microbial Growth and Substrate Utilization

$$R = \frac{R_{\max}[S]}{k_m + [S]} \quad \text{Michaelis - Menten Equation}$$

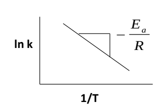
$$\mu = \frac{\mu_{\max}[S]}{k_s + [S]} \quad \text{Monod's Equation}$$

$$q = \frac{q_{\max}[S]}{k_s + [S]}$$

$$q = q_m \text{ for } S \succ\succ k_s$$

$$q = [q_{\max} / k_s]S = kS$$

Effect of Temperature on Rate Constant (Arrhenius Equation)



$$-\frac{E_a}{R}$$

$$\frac{\partial(\ln k)}{\partial T} = \frac{E_a}{R}\frac{1}{T^2}$$

$$or \ \ln k = -\frac{E_a}{R}\frac{1}{T} + \ln B \text{ (constant)}$$

Integrating between the two temperature limits (T_1 & T_2)

$$\ln\frac{k_1}{k_2} = \frac{Ea(T_2 - T_1)}{RT_1T_2}$$

$$\ln\frac{k_2}{k_1} = Const.(T_2 - T_1)$$

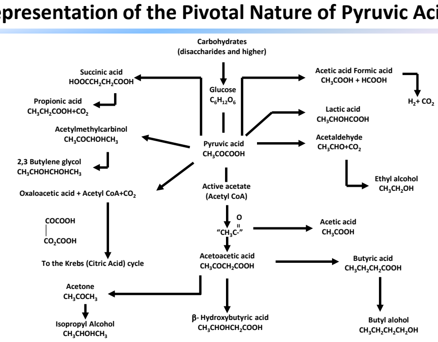
$$or \ \frac{k_2}{k_1} = e^{const(T_2 - T_1)}$$

$$= \Theta^{(T_2 - T_1)}$$

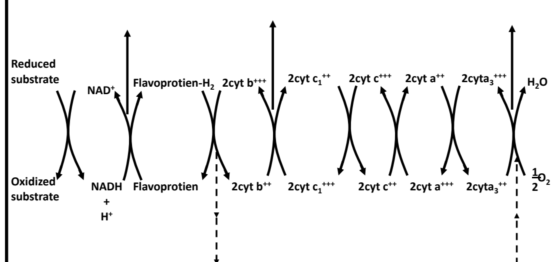
E_a → For biological WWT Process generally will fall within the range 2000-20,000 cal/mole or 8400-8,4000 J/mole
R → 8.314 J/mole/°K

Assumptions:
1) volumetric flow rate constant
2) no evaporation (isothermal)
3) reaction occurring within the boundaries
4) complete mixing
5) reaction order

Representation of the Pivotal Nature of Pyruvic Acid



Heterotrophic Aerobic Bacteria Energy for synthesis of ATP



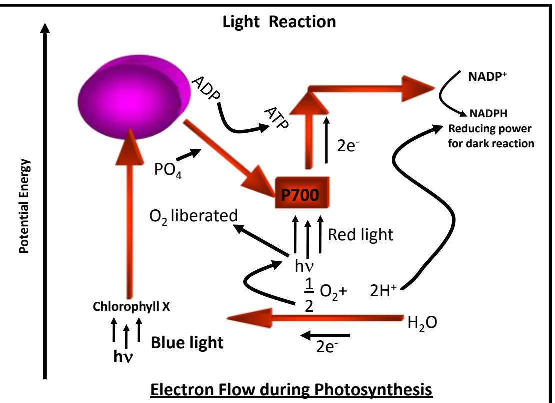
Electron Transport System Common to Most Bacteria

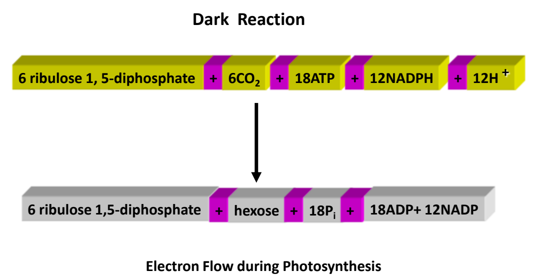
Applications

- Selection of Microorganisms
  - Heterotrophic or Autotrophic
  - Aerobic or Anaerobic
  - Chemosynthetic or Photosynthetic
- Growth Rate/Condition of Microbes
  - High Growth Rate or Auto Oxidation/Endogenous Phase
- Physical and Chemical Environment
  - Temperature, pressure
  - pH, nutrition, toxic substances
- Housing and Mixing
  - Suspended/immobilized or fixed or attached, homogenous/heterogeneous, stratified/un-stratified, uniform/non-uniform, steady/unsteady
- Ecology
  - Competition, symbiosis, predation, etc.
- System Performance Criteria
  - Removal, sludge production, gas production, energy requirement, etc.

Applications

- Suspended Growth Systems - Activated Sludge Process and its Modifications
- Immobilized/Attached Growth or Fixed Film Systems - Trickling Filter/Rotating Biological Contactors

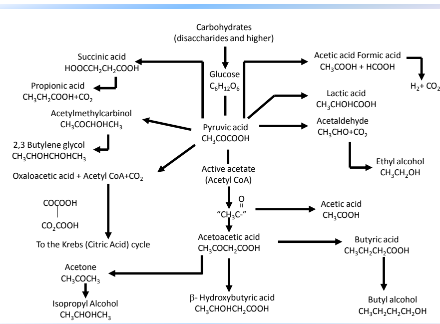




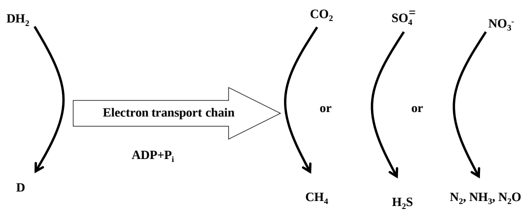
Applications

- **Oxidation Ponds** – Suspended Growth Systems
- **Facultative Ponds** – Suspended Growth Systems
- **Anaerobic Ponds** – Suspended Growth Systems

Representation of the Pivotal Nature of Pyruvic Acid

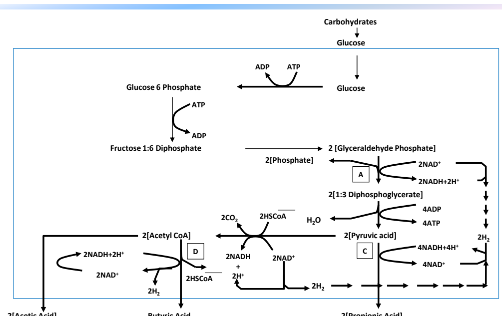


Electron Transport Chain for Anaerobic Respiration



Applications

- Anaerobic Systems – Denitrification
- Anaerobic Systems – Sulphate Reduction
- Anaerobic Systems – Methanogenesis



Standard Gibbs Free Energy Values (ΔG°) for Conversion of Ethanol, Propionate and Butyrate to Methane

Source: McCarty and Smith, 1986

S No.		Reaction	ΔG°(KJ)
1	**Ethanol Conversion**		
	Ethanol	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.65
	Hydrogen	$2H_2 + ½\ CO_2 \rightarrow ½\ CH_4 + H_2O$	-65-37
	Acetate	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-35.83
	Net	$CH_3CH_2OH \rightarrow 3/2\ CH_4 + ½\ CO_2$	-91.55
2	**Propionate Conversion**		
	Propionate	$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + 3H_2 + CO_2$	+71.87
	Hydrogen	$3H_2 + ¾\ CO_2 \rightarrow ¾\ CH_4 + 3/2\ H_2O$	-98.06
	Acetate	$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$	-35.83
	Net	$CH_3CH_2COO^- + H^+ + ½\ H_2O \rightarrow 7/4\ CH_4 + 5/4\ CO_2$	-62.22

Standard Gibbs Free Energy Values (ΔG°) for Conversion of Ethanol, Propionate and Butyrate to Methane

Source: McCarty and Smith, 1986

S No.		Reaction	ΔG°(KJ)
3	**Butyrate Conversion**		
	Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H_2 + H^+$	+48.30
	Hydrogen	$2H_2 + ½\ CH_4 + H_2O$	-65.37
	Acetate	$2\ CH_3\ COO^- + 2\ H^+ \rightarrow 2CH_4 + 2CO_2$	-71.66
	Net	$CH_3CH_2CH_2COO^- + H_2O + H^+ \rightarrow 5/2\ CH_4 + 3/2\ CO_2$	-88.73

Some Redox Half-reactions for Degradation of Selected Organics during Anaerobic Treatment of Industrial, Municipal, and Agricultural Wastes

Source: Thauer *et al*, 1977

S No.	Reaction	ΔG°(KJ)
Oxidations (electron donating reactions)		
1	Propionate → Acetate:	
	$CH_3COO^- + 3\ H_2O \rightarrow CH_3COO^- + H^+ + HCO^-_3$	+76.1
2	Butyrate → Acetate:	
	$CH_3CH_3COO^- + 2H_2O \rightarrow 2\ CH_3COO^- + H^+ + 2H_2$	+48.1
3	Ethanol → Acetate:	
	$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	+9.6
4	Lactate → Acetate	
	$CH_3CHOHCOO^- + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 2H_2$	-4.2
5	Acetate → Methane:	
	$CH_3COO^- + H_2O \rightarrow HCO^-_3 + CH_4$	-31.0

Some Redox Half-reactions for Degradation of Selected Organics during Anaerobic Treatment of Industrial, Municipal, and Agricultural Wastes

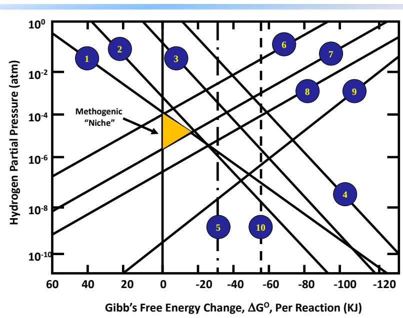
Source: Thauer *et al*, 1977

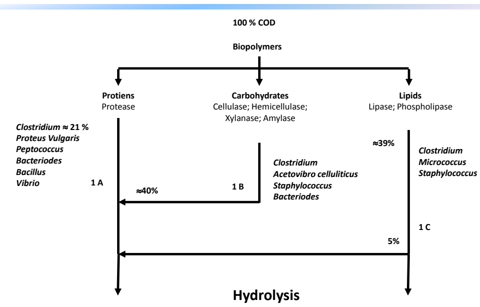
S No.	Reaction	ΔG^O(KJ)
Respiratory (electron accepting reactions)		
6	HCO_3^- → Acetate:	
	$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$	-104.6
7	HCO_3^- → Methane:	
	$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$	-135.6
8	Sulfate → Sulfide:	
	$SO_4^{-2} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$	-151.9
9	Sulfite → Sulfide	
	$SO_3^{-2} + 3H_2 + H^+ \rightarrow HS^- + 3H_2O$	-286.5
10	$CH_3COO^- + SO_4^{-2} + H^+ \rightarrow 2HCO_3^- + H_2S$	-59.9

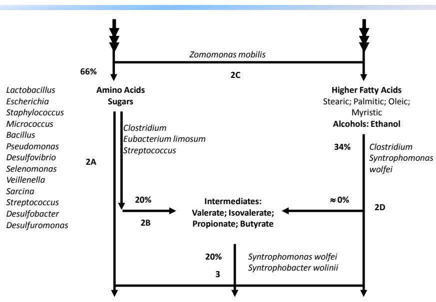
Some Redox Half-reactions for Degradation of Selected Organics during Anaerobic Treatment of Industrial, Municipal, and Agricultural Wastes

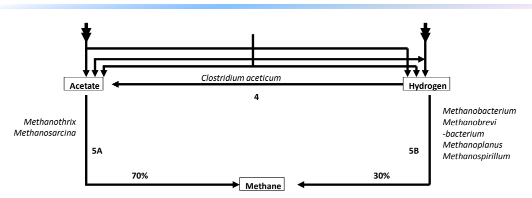
Source: Thauer *et al*, 1977

S No.	Reaction	ΔG^O(KJ)
Respiratory (electron accepting reactions)		
11	Nitrate → Ammonia:	
	$NO_3^- + 4H_2 + 2H^+ \rightarrow NH_4^- + 3H_2O$	-599.6
12	$CH_3COO^- + NO_3^- + H^+ + H_2O \rightarrow 2HCO_3^- + NH_4$	-511.4
13	Nitrate → Nitrogen gas:	
	$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O$	-1120.5









Methanogenesis

Percentages indicate substrate flow (stoichiometrically) in the form of COD. Only the net flow of substrate (degradation minus biomass formed) through cell external pools is indicated. Numbers in boxes identify different pathways.

Breakdown of Organic Polymers alongwith Bacterial Species involved in Methanogenesis. Sources: Gujer and Zehnder, 1983 and Stronach et al. , 1986).

Control of Microorganisms:
Principles and Physical Agents

- **Overview:**
  - Effective management of microorganisms in the laboratory, the home, the hospital and the industrial setting depends upon a knowledge of how to control (i.e. kill, inhibit, or remove) microorganisms in an environment.
  - Various physical and chemical agents can be used to keep microorganisms at acceptable levels.
  - Selection of the best agent depends in part on whether you want to kill or remove all of the microbes present, kill only certain types, or merely prevent those already present from multiplying.
  - Some familiar uses of physical agents to control microorganisms include the thorough cooking of poultry and meat to kill *Salmonella* bacteria, and the pasteurization of milk to destroy bacteria that can cause tuberculosis and typhoid fever.

Control of Microorganisms:
Principles and Physical Agents

- Fundamentals of Microbial Control
  - Substances that either kill microorganisms or prevent their growth are called *antimicrobial* agents. More specifically, these are *antibacterial*, *antiviral, antifungal,* and *antiprotozoan* agents, depending on the kind of microorganism affected.
  - Antimicrobial agents that kill microorganisms are called as microbicidal agents. The names bactericidal, virucidial, and fungicidal indicate the type of microorganism killed.
  - Killing all the microorganism present in a material including any spores, is called sterilization.
  - Agents that merely inhibit the growth of microorganisms are called microbiostatic agents. Again, more specific names can be used, such as bacteriostatic or fungistatic.
  - Antimicrobial agents may be either physical agents or chemical agents.

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Control of Microorganisms:
Principles and Physical Agents

- Pattern of death in a Microbial Population (FIG)
- Conditions that Affect Antimicrobial Activity
  - When microbicidal agents are used for some practical application, there can be great variation in the conditions affecting each situation.
  - Some important variables to consider when accessing the effectiveness of a microbicidal agent are:
    1. Size of microbial population. Large populations take longer to kill than small populations. FIG
    2. Intensity or concentration of the microbicidal agent. The lower the intensity or concentration, the longer it takes to kill a microbial population. Fig
    3. Time of exposure to the microbicidal agent. The longer the time, the greater the number of cells killed.
    4. Temperature at which the microorganisms are exposed to the microbicidal agent. In general, the higher the temperature, the more quickly a population is killed. Fig
    5. Nature of the material containing the microorganisms.
    6. Characteristics of the microorganisms which are present. Microorganisms vary considerably in their resistance to physical and chemical agents. For example, many Gram-positive species are more resistant to heat than Gram-negative species; some chemicals are more effective against Gram-positive species than they are against Gram-negative species.

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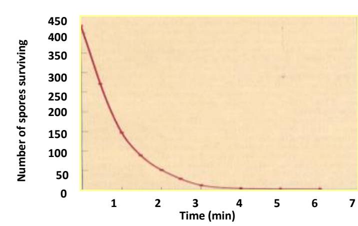
The arithmetic death curve of the bacterial spores exposed to a 5% phenol solution at a constant temperature illustrates that the spores in the population die over a period of time.



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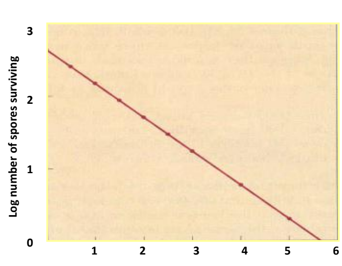
The logarithmic death curve is based on the same data as the preceding curve. Data expressed in the manner reveal a consistent increment of the deaths per unit time.



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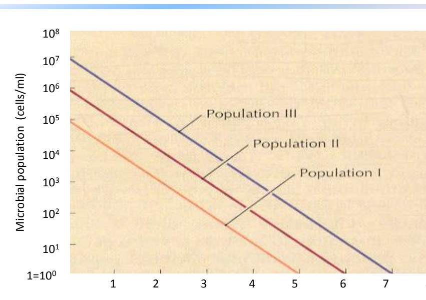
Graph showing death rates of three different microbial populations exposed to same microbicidal agent. Population I is the smallest and is killed in the shortest period of time. Populations II and III each require a longer period of time to be killed because the initial populations are larger



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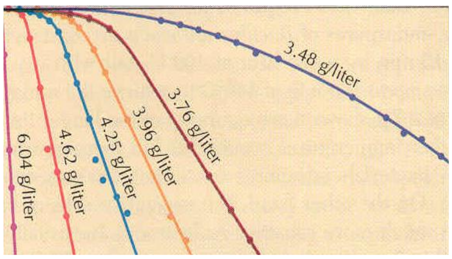
Effect of concentration of an anti-microbial agent on bacterial death. In this experiment, *Escherichia coli* was exposed to various concentrations of phenol at 35 ^{o}C. The number of survivors, expressed logarithmically, is plotted against time.

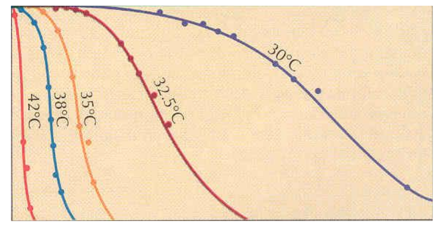
Log number of survivors per ml



Time (min)

An increase in temperature decreases bacterial survival when the concentration of the anti-microbial agent remains the same. In this experiment, *Escherichia coli* was exposed to phenol at a concentration of 4.62 g/liter at temperatures between 30 and 42 ^{o}C. The number of survivors, expressed logarithmically, is plotted against time.

Log number of survivors per ml



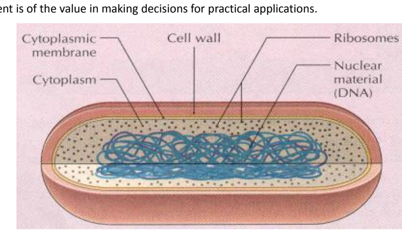
Time (min)

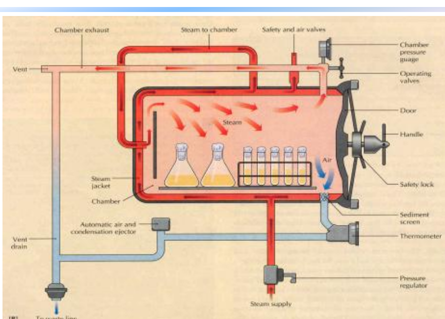
Control of Microorganisms:

Principles and Physical Agents

- Mechanisms of Microbial Cell Damage Fig
- High temperatures
  - The use of high temperatures is one of the most effective and widely utilized means of killing microorganisms. Heat may be applied in either a moist condition (steam or water) or in a dry condition. The most extreme use of high temperatures to kill microorganisms is incineration (burning)
- Moist Heat Fig
- Dry heat
- Low Temperatures

Antimicrobial agents inhibit or kill microorganisms by damaging certain structures of the cells, such as the cell wall or the cytoplasmic membrane, or substances within the cytoplasm, such as enzymes, ribosomes, or nuclear material. Knowledge of the mode of action of an anti microbial agent is of the value in making decisions for practical applications.

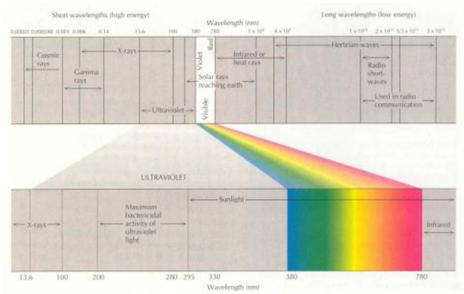




Control of Microorganisms:

Principles and Physical Agents

- **Radiation**
  - Electromagnetic radiation is energy in the form of electromagnetic waves transmitted through space or through a material. Electromagnetic radiation is classified according to its wavelength, with radio waves having the longest wavelength and cosmic rays having the shortest.
  - The energy content of the radiation is inversely related to the wavelength: shorter the wavelength, the greater the energy content.
  - High-energy radiation includes gamma rays, x-rays, and ultravoilet lights. Theses can kill living cells, including microorganisms.
  - Some form of electro magnetic radiation ionize molecules, while others do not.



Control of Microorganisms:
Principles and Physical Agents

- Radiation
  - Ionizing radiation
    - High energy electron beams, gamma rays, and x-rays have sufficient energy to cause ionization of molecules: they drive away electrons and split the molecules into atoms or groups of atoms.
  - Non ionizing radiation
    - Ultravoilet (UV) radiation has a wavelength range of 136 to 400 nanometers (nm).
    - Rather than ionize a molecule, UV light excites its electron causing the molecule to react differently from nonirradiated molecules.
- Filtration
  - Membrane filters
- Desiccation

Control of Microorganisms
Chemical Agents

Overview

- There are hundreds of different chemical products available for the control of microorganisms.
- Certain antimicrobial chemicals kill microorganisms, while other inhibit their growth.
- Some can do either, depending on the concentration at which they are used.
- Some are active against a large number of species and are characterized as having a broad spectrum of activity, while other chemical agents may affect only a few species.
- There is not a single chemical agent that is optimal for all the purposes.

Control of Microorganisms
Chemical Agents

Terminology of Chemical Antimicrobial Agents

- **Sterilant:** Sterilization is the process of destroying or removing all forms of microbial life from an object or a specimen. Thus a sterile item is one which is free of all living organisms, and a sterilant is a chemical agent that accomplishes sterilization.
- **Disinfectant:** A disinfectant is a chemical substance that kills the vegetative forms of microorganisms that can cause disease but does not necessarily kill there spores. The term normally refers to substances used on inanimate objects. Disinfection is the process of using such an agent to destroy infections microorganisms
- **Germicide:** A chemical agent that kills the vegetative forms of microorganisms, but not necessarily their spores, is called a germicide. In practice, it is almost synonymous with a disinfectant; however the microorganisms killed by a germicide are not necessarily disease-producing microbes.

Control of Microorganisms
Chemical Agents

Terminology of Chemical Antimicrobial Agents

- **Antiseptic:** An antiseptic is a chemical agent, usually applied to the surface of the body, that prevents microorganisms from multiplying.
- **Sanitizer:** Public health guidelines mandate that, in certain settings, microbial populations should not exceed specific numbers. Compliance with this rules is accomplished by using a sanitizer, an agent that kills 99.9 percent of microorganisms contaminating an area.

Control of Microorganisms
Chemical Agents

Characteristics of an Ideal Chemical Agent:

- Antimicrobial activity
- Solubility
- Stability
- Lack of toxicity
- Homogeneity
- Minimum inactivation by extraneous material
- Activity at ordinary temperatures
- Ability to penetrate
- Material safety
- Deodorizing ability
- Detergent ability
- Availability and low cost

Control of Microorganisms
Chemical Agents

Major groups of Disinfectant and Antiseptics

- Chemical substances used for disinfection or antisepsis are divided into several major groups: Phenol and phenolic compounds, alcohols, the halogens iodine and chlorine, heavy metals and their compounds, and detergents
  - **Phenol and Related Compounds:**
    - Phenol, also called carbolic acid, has the distinction of being one of the first chemical agents used as an antiseptic
  - **Mode of action of phenol and related compounds:**
    - Phenol and phenolic compounds damage microbial cells by altering the normal selective permeability of the cytoplasmic membrrane, causing leakage of vital intracellular substances.
    - These chemicals also denature and inactivate proteins such as enzymes.
    - They may be either bacteriostatic or bactericidal, depending on the concentration used.

Control of Microorganisms
Chemical Agents

Major groups of Disinfectant and Antiseptics

- **Alcohols:**
  - In concentrations between 70 and 90 %, solutions of ethyl alcohol (ethanol), CH_3CH_2OH, are effective against the vegetative forms of microorganisms. But ethyl alcohol can not be relied upon to sterlize an object because it does not kill bacterial endospores.
  - Methyl alcohol, or methanol (CH_3OH) $\rightarrow$ X ?
- **Mode of action of alcohols:**
  - Alcohols are protein denaturants, which accounts to a large extent for their antimicrobial activity.
  - Alcohols are also lipid solvent, thus damaging the lipid structure within microbial cell membranes.
  - In addition, some of their effectiveness as surface disinfectant can be attributed to their cleansing or detergent action, which helps in the mechanical removal of microorganisms.

Control of Microorganisms
Chemical Agents

Major groups of Disinfectant and Antiseptics

Halogens

- **The halogens are strong oxidizing agents and by virtue of this property are highly reactive and destructive to vital compounds within the microbial cell.**
- **Iodine and Iodine Compounds**
  - **Mode of action of iodine and its compounds.**
    - **A strong oxidizing agent, iodine can destroy essential metabolic compounds of microorganisms through oxidation. The ability of iodine to combine with the amino acid tyrosine results in the inactivation of enzymes and other proteins.**

Control of Microorganisms
Chemical Agents

Major groups of Disinfectant and Antiseptics

Halogens

- Chlorine and chlorine compounds:
  - Mode of action of chlorine and its compounds:
    - The antimicrobial action of chlorine and its compounds is due to the hypochlorous acid (HClO) formed when free chlorine is added to water:

$$Cl_2 + H_2O \rightarrow HCl + HClO$$

    - When added to water, hypochlorites and chloramines undergo hydrolysis, giving rise to hypochlorous acid. This acid undergoes further change, giving rise to nascent oxygen (O):

$$HClO \rightarrow HCl + O$$

    - Nascent oxygen is a powerful oxidizing agent that can severely damage vital cellular substances. Chlorine may also combine directly with cellular proteins and destroy their biological activity.

Control of Microorganisms
Chemical Agents

Major groups of Disinfectant and Antiseptics

- Heavy Metals and Their Compounds
- Detergents

Control of Microorganisms
Chemical Agents

Chemical Sterilants

- Chemical sterilants are particularly useful for the sterilization of heat-sensitive medical supplies, such as plastic blood transfusion or donor sets, plastic syringes, and catheterization equipment.
- The major chemical sterilants in use are
  - Eethylene oxide
  - β - propiolactone
  - Glutaraldehyde
  - Formaldehyde.